2. Stability Data and Expiration Dating

Stability of [I-123]5-IA preparation was evaluated by high performance liquid chromatography after its storage in the final dosage form in the sterile container-closure system (as supplied for administration) for up to two days at +4°C (**Error! Reference source not found.**). The labeled product retained radiochemical purity within specifications for up to 49 hours after formulation. A conservative expiration time of 36 hours after calibration was placed on the product.

3. Bacterial Endotoxin (LAL) Pyrogen Test Limits

Based on the guideline to limit patient exposure to endotoxins (pyrogens) of NMT 5 eu/kg/h (endotoxin units per kilogram per hour) for infusion solutions (USP XXIV 2000), the estimated maximum concentration for an entire 10 mL dose given to a 70 kg individual in a single dose would be 5 x 70 = 350 eu / (6–10 mL) or about 35–55 eu/mL. Setting a specification an order of magnitude below that, we arrive at 5 eu/mL for the final dosage form. At this endotoxin concentration, a positive control diluted 1:4 (i.e. final concentration 1.25 eu/mL) can be expected to gel reliably at 20–30 min incubation (Zulstra et al 1997); we chose 30 min for a margin of safety.

3. Pyrogen Test (LAL Bacterial Endotoxin Test)

a) Materials

- LAL test kit (e.g., Endosafe Inc., Charleston, SC or Associates of Cape Cod Inc, MA)
- Endotoxin standard, lyophilized
- Sterile water for irrigation, USP or isotonic sterile sodium chloride injection, USP (0.9% NaCl)
- Pyrogen-free test tubes (if not provided with kit)
- Micropipets and tips
- 0.5 mL Tuberculin syringes
- Water bath, 35–39°C

Table 1. Dilution of standard endotoxin for working positive control standard

	Positive Control	Endotoxin Concentration
Sample/ Standard	Dilutions	eu/mL
CSE	Reconstitute one vial with *qs* water (see package insert)	1,000
1.25	50 µL **CSE** + 10 mL sodium chloride injection, USP	1.25
5	5 µL **CSE** + 995 µL H_2O	5.0
T	Test Article (Radiopharmaceutical)	

b) Procedure

- *Stock Endotoxin Solution (Control Standard Endotoxin, **CSE**).* Reconstitute endotoxin standard with H_2O to obtain a solution with 1,000 eu/mL (endotoxin units per milliliter). *Example*: if the standard vial contains 500 ng at 10 eu/ng (total 5,000 eu/vial), add 5 mL H_2O to the vial. Add the H_2O and vortex vigorously for 1 min. Label the vial with the date and volume reconstituted. Store at 0–4°C. Discard after four weeks.
- *Positive Control*. Prepare within five days of test. Store at 0–4°C. Dilute as shown in Table 1 for 1.25 or 5 eu/mL standard (vortex each sample vigorously). Alternatively use single lyophilized positive control test vials containing, from an original, 0.2 mL LAL and 1.0 eu/mL with the corresponding LAL test kits from Associates of Cape Cod Inc.
- *Sample Preparation*. Add 2 µL saturated $NaHCO_3$ solution to test sample to adjust pH.

- *Test Procedure*. Add sample to a test kit vial through the septum with syringe and mix well.

Samples ≥10 mL): Add 0.2 mL test sample. Dilution Factor = 1.

Samples <10 mL: Add 0.1 mL test sample & 0.1 mL sodium chloride injection, USP. Dilution Factor = 2

Or 0.05 mL test sample & 0.15 mL sodium chloride injection, USP. Dilution Factor = 4

Positive Controls. Add 0.2 mL of **1.25 eu/mL** standard *or* dilute **5 eu/mL** standard 1:4.

Record dilution factor(s) on the test sheet.

- Incubate tubes for 30 min. Do not disturb the samples during the incubation.

- In one smooth motion, invert each tube and note gel formation. The test sensitivity equals the concentration of the positive control sample multiplied by the dilution factor. If the test sample does not gel, report the result as less than the test sensitivity. If the test sample gels, report as ≥ test sensitivity.

4. Sterility Test

c) Materials

- Culture media:
  Fluid Thioglycollate Broth (large tubes)
  Soybean-Casein Digest (small tubes)
- Empty sterile vial.
- Sterile sodium chloride injection, USP vial
- Sterile syringe, 1–10 cc, and needles 20–23 g
- Alcohol pads

d) Procedure I

1. Aseptically remove a 25 µL aliquot from the dose vial and place in a sterile vial containing sterile normal sodium chloride injection, USP.
2. Submit the vial to the NIH microbiology Lab for determining the sterility of the radiopharmaceutical.
3. *In Case of Positive Result*. If growth is reported: a) notify the PI; b) ask for the identity of the organism from the microbiology lab; c) file report on the investigation and follow-up results in the GMP investigations file.

e) Procedure II

1. Perform inoculations in a laminar flow hood (LFH) with HEPA filter.
2. Prepare culture tubes of Fluid Thioglycollate and Soybean-Casein Digest media; label each set with the date or lot # and the designation: (**O**), (+), ($\mathbf{T_1}$), ($\mathbf{T_2}$), ($\mathbf{T_3}$).
3. Loosen caps of tubes. Treat tubes as follows:
4. (**O**) Control Open tube: remove cap during remainder of process.
5. ($\mathbf{T_x}$) Test article Transfer 1–2 drops of test article (in triplicate) into each tube.
6. (+) Positive Spit into cap while facing away from LFH. (This sample is inoculated last, outside the LFH).
7. Close screw cap closures and mix all tubes by inverting.
8. Place Fluid Thioglycollate tubes in incubator at 30–35°C; store Soybean-Casein tubes at room temperature (20–25°C).
9. Read tubes at 7 and 14 days after inoculation. A positive result is seen as definite turbidity or flocculent solid in the liquid (faint flecks of protein denaturation, if visible also in the negative control, don't count). For sterility, all tubes except the positive control must be clear and the positive control must be turbid.
10. *In Case of Positive Results.* If growth is observed in the test samples: a) notify the PI; b) save the culture tube for isolation and identification of the organism by the microbiology lab; c) file report on the investigation and follow-up results in the GMP investigations file.

5. Strength (Radiotracer)

Gamma ionization chamber ("Dose calibrator").

Direct Method. Draw amount to be measured in a 10-cc plastic syringe in a volume of 8-10 mL. Place the syringe in the syringe holder bracket and read directly at the setting for I-123.

Indirect Method. Measure sample in a container and at a geometry that has previously been calibrated at each geometry used. Multiply the reading by the correction factor determined for the geometry used.

Studies of the pharmacology and toxicology of the agent were performed by our collaborators at the Addiction Research Center, Intramural Research Program, National Institute of Drug Abuse. In particular, DB Vaupel, Ph.D. performed the rodent studies; and NIDA arranged for the Ames test to be performed by MPI Research (54943 North Main Street, Mattawan, MI 49071-9399). R.B. Innis, M.D., Ph.D. and M. Fujita, M.D. Ph.D. arranged for in vitro micronucleus assay to be performed by Covance Laboratories, Inc. (9200 Leesburg Pike Vienna, VA 22182). The results are summarized here and described in more detail in Section III.B. In the Ames test, 5-I-A-85380 was weakly positive in the Bacterial Reverse Mutation assay as one of five tester strains demonstrated a positive response, and then only in the presence of Aroclor-induced rat liver S9. Positive responses required doses greater than 1,000 μg per plate. The clastogenic potential of 5-I-A-85380 was also assessed by measuring the frequency of micronucleated binucleated CHO cells treated with and without metabolic activation. Rat liver S9 fraction was used as a metabolic activation system. 5-I-A-85380 did not show positive reaction at any tested concentration up to 150 ng/mL. A comparison of the convulsion-inducing properties of 5-I-A-85380 to those of nicotine in CD-1 mice demonstrated that 5-I-A-85380 produced clonic or clonic and tonic convulsions. The ratio of ED50 values for eliciting seizures by the intravenous route demonstrated that 5-I-A-85380 (7.1 μmol/kg) was only one-fifth as potent as nicotine (1.4 μmol/kg). Acute toxicity studies were performed by intravenous administration of 0.3, 3, 15, 30, and 150 nmol/kg in CD-1 mice. The lowest dose of 5-I-A-85380 associated with a noticeable physiological effect was 30 nmol/kg, at which eight of ten animals showed Straub tail. There was no evidence of gross pathological or histopathological changes in this dose range. Subacute – chronic toxicities were assessed by 14-day repeated subcutaneous administration of 0.3, 3, and 30 nmol/kg. These doses did not cause any behavioral effects and were associated with a minimal incidence of gross pathology. An assessment of the cardiovascular, locomotor and temperature properties in unanesthetized rats demonstrated that 5-I-A-85380 (5.2 to 174 nmol/kg, iv) resembled nicotine (20 to 400 nmol/kg, iv) by producing rapid, dose-dependent increases in systolic pressure, diastolic pressure, heart rate, and locomotor activity. For maximal or near maximal doses, the durations of action for nicotine and 5-I-A-85380 were 1 h and 2 h, respectively. Doses that were effective in producing 10% increases blood pressure and heart rate ranged between 5 and 10 nmol/kg, iv. In contrast to the similarity in the preceding effects, 5-I-A-85380 dose-dependently elevated core body temperature whereas nicotine reduced temperature.

The mass dose of 15 mCi [I-123]5-I-A-85380 in a subject weighting 70 kg was calculated to be 43 pmol/kg body weight from the molecular weight of 290 and assuming a conservative specific activity of 5,000 Ci/mmol. **This calculated mass dose is at least**

100 times smaller than the minimal effective dose in the experiments reported by Dr. Vaupel, MPI Research, and Covance described below and appendixes.
Furthermore, the specific activity of our product is > 5,000 Ci/mmol (see section II). Therefore, this calculation of mass dose of [I-123]5-I-A-85380 in humans is an upper limit for a 70 kg subject.
In summary, we expect the injection of 15 mCi of non-carrier-added [I-123]5-I-A-85380 to cause no pharmacological effects in humans.

A. Bacterial Mutation and Animal Toxicology Studies

MPI Research and D.B. Vaupel, PhD at the NIDA Brain Imaging Center have provided data on bacterial reverse mutation assays (Ames test) and toxicology studies in CD-1 mice and Sprague-Dawley rats, respectively. Under the prior IND #61,156, R.B. Innis, M.D., Ph.D. and M. Fujita, M.D., Ph.D. obtained results of in vitro microwell micronucleus screening assay from Covance Laboratories Inc. The complete reports are in the Appendix and summary statements are provided below. The materials used for these studies were prepared and characterized by the methods described in the prior IND #61156 and the results were also reported in the prior IND.

1. Bacterial Mutation Studies (Ames Test)

The test article, 5-I-A-85380, was tested in the Bacterial Reverse Mutation Assay using *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *E. coli* teter strain WPR *uvr*A in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases using the plate incorporation method. In the first phase, the preliminary toxicity assay was used to establish the dose range for the mutagenicity assay. The maximum dose tested was 5,000 µg per plate, achieved using a concentration of 50 mg/mL in dimethyl sulfoxide and a 100-µL plating aliquot. Neither precipitate nor appreciable toxicity was observed. Based on findings of the toxicity study, the maximum dose plated in the mutagenicity assay was 5,000 µg per plate. The second phase, the mutagenicity assay, evaluated the mutagenic potential of 5-I-A-85380 by applying 100, 333, 1,000, 3,333, and 5,000 µg per plate. A positive response was observed for the 3,333 and 5,000 µg doses in the TA1535 tester strain only in the presence of Aroclor-induced rat liver S9 but not in other tests. These doses produced 4.5- and 4.1-fold maximum increases in the mean number of revertants over the mean value of the respective control vehicle. Because a three-fold increase was the criterion for a positive response, the increases with 3,333 and 5,000 µg doses were weakly positive. Neither precipitate nor appreciable toxicity was observed. Under the conditions of this study, 5-I-A-85380 was weakly positive in the Bacterial Reverse Mutation Assay.

The dose of 15 mCi [I-123]5-I-A-85380 (> 5,000 Ci/mmol) in human subjects (body weight: 70 kg; dose: 43 pmol/kg) is at least 0.8×10^9-fold lower than the highest dose that did not cause mutagenic effect (1,000 µg per plate, 10 mg/mL = 0.034 mol/kg body weight) in this study.

2. In Vitro Microwell Micronucleus Screening Assay

In the communication from the FDA dated 11/16/00 (PHARMACOLOGY AND TOXICOLOGY COMMENTS, #4) on the prior IND #61,156, FDA recommended in vivo mouse micronucleus assay because the Ames tests submitted in the originally

application on 10/25/00 showed positive reactions as described above. However, the reactions were weakly positive. Therefore, we performed in vitro micronucleus assay and reported in the prior IND.

In brief, the clastogenic potential of 5-I-A-85380 was assessed by measuring the frequency of micronucleated binucleated CHO cells treated with and without metabolic activation. The tested concentrations were 1.18, 2.35, 4.70, 9.40, 18.8, 37.5, 75.0, and 150 ng/mL. In addition to the assays with 5-I-A-85380, cyclophosphamide (5 μg/mL) was used as positive control. Rat liver microsomes (S9 fraction) were used as a metabolic activation system.

5-I-A-85380 did not show positive reaction at any tested concentration. **The highest concentration tested in the in vitro micronucleus assay is 12,000 times greater than the mass dose of 15 mCi (specific activity > 5,000 Ci/mmol) assuming that [I-123]5-I-A-85380 is uniformly distributed in the entire body (70 L). Therefore, we think the mass dose of 15 mCi [I-123]5-I-A-85380 is highly safe in terms of mutagenicity.**

3. Convulsive Dose ED_{50} Study in the Mouse

A Convulsant ED_{50} Toxicity Study was conducted at the NIDA Brain Imaging Center using intravenously administered 5-I-A-85380 to determine dosages for producing physiological signs of acute toxicity. This is an appropriate toxicity model since convulsions are well-established effect of nicotine itself. 5-I-A-85380 was tested in doses of 2, 3.5, 6, 11 and 20 μmol/kg, and nicotine, which served as the standard, was tested in doses of 1, 1.25, 1.5, and 2 μmol/kg. Ten mice (5 male and 5 female) were tested at each dose except for 20 μmol/kg 5-I-A-85380 for which n = 6. Following the administration of the drugs via the tail vein, mice were observed for a 1-h period.

The calculated ED_{50} for producing convulsions in male and female CD-1 mice was 7.1 μmol/kg with 95% confidence limits of 5.2 and 9.7 μmol/kg (Fig, appendix), and the calculated was ED_{10} was 4.5 μmol/kg. The development of convulsions was dose-dependent, and seizure activity was primarily characterized as clonic or clonic and tonic accompanied by loss of the righting reflex. At the highest dose tested (20 μmol/kg), all animals (3 male and 3 female) died within 15 –75 seconds. Rapid forepaw treading movements and labored respiratory movements, sometimes accompanied by gasping, were commonly observed with lower subconvulsant doses. The ED_{50} to produce convulsions for nicotine was 1.4 μmol/kg with 95% confidence limits of 1.2 and 1.7 μmol/kg. In addition to eliciting clonic convulsions, "running fits" (extremely rapid running around the perimeter of the cage) were frequently observed at 1.5 and 2 μmol/kg. Additionally, both 5-I-A-85380 and nicotine reduced locomotor activity and produced motor incoordination, Straub tail, and respiratory distress at lower subconvulsant doses. 5-I-A-85380 produced a marked 9-10 °C decrease in rectal temperature over a 1-h period, whereas nicotine had no effect on temperature. The sterile sodium chloride injection, USP vehicle produced none of these effects.

Taken together, depression of locomotor activity, motor incoordination, respiratory distress, and convulsions likely constitute the toxic effects of 5-I-A-85380. These effects are similar to the toxic effects of nicotine. Based on the ratio of ED_{50} values for producing convulsions, nicotine was five-fold more potent than 5-I-A-85380. **The dose of 15 mCi [I-123]5-I-A-85380 (> 5,000 Ci/mmol) in human subjects (body**

weight: 70 kg; dose: 43 pmol/kg) is at least 1 x 10^5 times lower than the ED_{10} (4.5 μmol/kg) for eliciting convulsions in mice.

4. Acute 2-Day Intravenous Toxicity Study of 5-I-A-85380 in the Mouse

The acute toxicity of 5-I-A-85380 was evaluated using single intravenous injections administered to CD-1 mice followed by a two-day observation period. Following drug administration (0, 0.3, 3, 15, 30, and 150 nmol/kg), behavior and physiological effects were observed for 1 h. Approximately 48 h after dosing, mice were euthanized, necropsies performed, and tissue samples harvested for histopathology. The study was conducted in two blocks of experiments in which groups of 5 male and 5 female mice of the CD-1 strain were used for each treatment condition. In each block of studies, mice were randomly assigned to treatments. Injections, observations, necropsies, and histopathology were performed under blind conditions.

5-I-A-85380 produced a dose-dependent appearance of Straub tail and a decrease in temperature; the lowest doses eliciting effects that differed significantly from the vehicle were 30 and 150 nmol/kg, respectively. Tremors and labored breathing appeared following the high dose, 150 nmol/kg, but there were no convulsions. There was a generalized loss in body weight over the 2-day period, but the effect was not attributable to treatment with 5-I-A-85380. None of the mice exhibited any gross pathology of the tail injection sites at the time of necropsy. Only one of 50 animals evaluated exhibited a possible 5-I-A-85380-related tissue lesion. Three mice that received 5-I-A-85380 treatment and two mice that received vehicle treatment died between 24 and 48 h after dosing. Histopathological examination of tissues from the five mice that died between 24 and 48 h after treatment did not identify any consistent pattern that would indicate 5-I-A-85380 as a causative factor in the deaths of the three mice treated with 5-I-A-85380 or any causative factor common to all five deaths. Some of these mice may have been stressed by accidental water deprivation attributed to malfunction of the automated watering system. Based on patterns of the incidence of deaths and tissue lesions, 5-I-A-85380 did not appear to produce gross pathological or histopathological changes.

Therefore, the dose of 15 mCi [I-123]5-I-A-85380 (> 5,000 Ci/mmol) in human subjects (body weight: 70 kg; dose: 43 pmol/kg) is at least 340 fold lower than the highest dose that did not cause noticeable effect (15 nmol/kg) in this study.

5. 14-Day Repeated Subcutaneous Dose Toxicity Study of 5-I-A-85380 in the Mouse

The toxicity of 5-I-A-853480 was evaluated following repeated daily subcutaneous injections to male and female CD-1 mice for a 14-day period. Three groups, consisting of 5 male and 5 female mice, received the test material in doses of 0.3, 3, and 30 nmol/kg. A fourth group of 5 male and 5 female mice received the sterile sodium chloride injection, USP vehicle, as the control group. All surviving mice were euthanized approximately 24 h after receiving the final treatment with 5-I-A-853480, and necropsies were performed. General behavior was assessed daily, 60 to 90 minutes after treatment using a 5-point rating scale based upon operational definitions. Changes in body weight over the 14-day period were recorded. Gross pathological examinations were completed on all animals, and complete histopathology assessments were performed on the vehicle and 30 nmol/kg treatment groups. Lesions observed in the remaining two treatment groups also were examined histopathologically.

5-I-A-85380 produced no abnormal effects on behavior throughout the 14-day period of treatment. There were no changes in body weight due to treatment with 5-I-A-85380, and there was a minimal incidence of gross pathology. Subcutaneous injections in the dorsal neck region were well tolerated, as there was only a single report of mild dermatitis at the injection site. The histopathological results of mice treated with 5-I-A-85380 were characterized by a low incidence of lesions that were mild in scope and observed commonly in mice. Therefore, **this study did not show toxicity of 5-I-A-853804 associated with a 14-day regimen of repeated subcutaneous injections.**

6. Cardiovascular studies in Unanesthetized Rats

The acute systolic and diastolic blood pressure, heart rate, locomotor activity, temperature effects of the test material 5-I-A-85380 administered by intravenous injection were compared to nicotine, which served as the standard drug, in unanesthetized rats using data telemetry. A group of 3 male and 3 female Sprague-Dawley rats were surgically prepared with telemetry transducers and a jugular vein catheter for drug administration. On test days, rats were acclimated to the test chamber for 60 to 90 min, either the drug or vehicle (sterile sodium chloride injection, USP for 5-I-A-85380 and sterile sodium chloride injection, USP adjusted to pH 4 for nicotine) was administered intravenously over 2 sec, and pharmacological effects were monitored continuously at 20 sec intervals for 2 h after dosing.

Cardiovascular effects of intravenously administered of 5-I-A-85380, tested in doses of 0, 5.2, 17, 52, and 174 nmol/kg, were characterized by increased systolic and diastolic pressure and stimulation of heart rate. Pressor effects had a rapid onset of action, becoming well developed in less than a minute. Within 3 min of the administration of 174 nmol/kg 5-I-A-85380, the respective peak increases in systolic and diastolic pressure averaged 43.4 ± 5.7 and 37.5 ± 3.3 mm Hg above their baseline values, which were 121.4 ± 4.4 and 88.7 ± 2.9 mm Hg, respectively. Average changes in systolic and diastolic pressures measured over the first 5 min were 19.5 ± 1.2 and 20.0 ± 0.9 mm Hg for this dose. Pressor effects of 52 and 174 nmol/kg returned to baseline levels within 2 h. By comparison, the lowest, 5.2 nmol/kg dose produced a peak increase of 17.2 ± 4.6 mm Hg and a mean increase of 9.8 ± 2.5 mm Hg in systolic and a peak increase of 15.3 ± 4.3 mm Hg and a mean increase of 8.4 ± 1.7 mm Hg in diastolic pressure during the first 5 min after 5-I-A-85380 administration. Both pairs of increases were significantly higher than the corresponding vehicle peak and mean increases, which were 3.7 ± 2.0- and 1.7 ± 1.8 mm Hg for systolic pressure and 2.9 ± 1.2- and 1.3 ± 1.1 mm Hg for diastolic pressure. Elevations in both systolic and diastolic pressure were dose-dependent and represented significant treatment effects of 5-I-A-85380 over the 2 h-period. The doses that caused a mean 10% increases in systolic and diastolic pressure occurring over the first 5 min after drug administration, were 11 (95% confidence limits: 2 – 23) and 5 (1 – 10) nmol/kg, respectively. This outcome was selected to reflect the minimally effective dose (MED), and this 10% increase in heart rate and blood pressure will be subsequently referred to as MED.

Tachycardia was a second major effect of 5-I-A-85380. The increases in heart rate were rapid in onset and most prominent during the first 5 min after administration; the duration of action was approximately 2 h for the high, 174 nmol/kg-dose. Mean increases in heart rate were generally dose-dependent, but equivalent peak levels of tachycardia

were produced by 52 nmol/kg (98.6 ± 24.0 beats/min) and 174 nmol/kg (99.7 ± 22.2 beats/min), although their times of appearance after administration were quite distinct, being 2.67 min and 19.33 min, respectively. In the first 5 min after drug administration, the mean increase in heart rate produced by 5.2 nmol/kg (25.0 ± 7.8 beats/min) represented a non-significant change, whereas the largest, significant mean increase in heart rate was produced by 52 nmol/kg (81.6 ± 20.9 beats/min). Male rats appeared to be more sensitive to the chronotropic effect of 5-I-A-85380, particularly to the 52- and 174 nmol/kg doses, but the findings were restricted to changes developing only within 10 min of drug administration. The MED, estimated from changes in heart rate developing during 0 to 5 min, was 5 nmol/kg (95% confidence limits: 0 – 17).

In addition, 5-I-A-85380 stimulated locomotor activity. Peak effects developed within 5 min, and the duration of action approached 2 h for the 174 nmol/kg dose. As with the effects on heart rate, male rats were more sensitive to the stimulant effects than females during the first 10 min after treatment. Body temperature also was dose-dependently increased by 5-I-A-85380. For the 174 nmol/kg dose, the hyperthermic effect averaged 0.60 °C over the 2 h-study period, with the peak increase of 0.94 °C occurring 75 min after drug administration.

Nicotine, tested in doses of 0, 20, 60, 201, and 400 nmol/kg, produced cardiovascular changes similar to those of 5-I-A-85380, except that the effects of nicotine had a shorter duration, typically disappearing within 1 h. Rapid elevations of systolic and diastolic pressures characterized the pressure response to intravenous nicotine particularly during the first 5 min. For the 400 nmol/kg-dose the peak increase in systolic pressure was 50.2 ± 9.8 mm Hg and for diastolic pressure it was 30.5 ± 3.4 mm Hg. Mean increases over the 0 to 5 min-period after nicotine averaged 21.2 ± 3.2 mm Hg for systolic pressure 19.4 ± 1.8 mm Hg for diastolic pressure. The net effect of nicotine was to stimulate heart rate. At the highest dose of nicotine tested, 400 nmol/kg, a brief episode of bradycardia (peak decrease of -21.6 ± 49.4 beats/min) preceded a rapid increase in rate and a subsequent peak increase of 78.6 ± 8.6 beat/min. During the period of bradycardia, two rats developed extreme reductions in heart rate (e.g. greater than 160 beats/min from a baseline mean of 279.5 ± 15.1 beats/min), which suggested the threshold of cardiotoxicity for nicotine was being approached. The resulting dose-response curves for heart rate stimulation during the 0 to 5-min and 5 to 10-min periods after nicotine were biphasic (inverted U). Locomotor activity was increased by nicotine for a period of 30 to 60 min, with the dose-response curve having an inverted U-shape during the initial 5 min, as stimulation following 400 nmol/kg was less than that of the 60 and 201 nmol/kg doses. The diminished efficacy of the high dose of nicotine to increase activity may reflect the onset of toxic effects, similar to that manifested on heart rate response. Hypothermia was the main effect of nicotine on temperature, and this effect was attributable only to the high, 400 nmol/kg-dose.

We concluded that the intravenous administration of 5-I-A-85380 produces dose-dependent increases in heart rate, systolic and diastolic blood pressure and locomotor activity, pharmacologic effects characteristic of nicotine. Based upon comparable ranges of active doses, onsets of action for these measures were rapid for both drugs, but the duration of action of 5-I-A-85380 exceeded that of nicotine in the rat. Estimates based on near maximal cardioactive doses, indicated durations of action of 2 h for 5-I-A-85380 and

1 h for nicotine in the rat. Signs of cardiotoxicity were observed with the 400 nmol/kg-dose of nicotine but not with the 174 nmol/kg-dose of 5-I-A-85380.

Although the lowest tested dose of 5-I-A-85380, 5.2 nmol/kg, showed statistically significant cardiovascular effects, the magnitude of changes was small and their durations of action were brief. Further, the dose of 15 mCi [I-123]5-I-A-85380 (> 5,000 Ci/mmol) in human subjects (body weight: 70 kg; dose: 43 pmol/kg) is at least 100 fold lower than lowest MED value (e.g., 5 nmol/kg) estimated from the cardiovascular study.